ORIGINAL ARTICLE

Retinol May Promote Fluorouracil-Suppressed Healing of Experimental Intestinal Anastomoses

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Objectives: To examine the effects of perioperative administration of fluorouracil on healing variables of intestinal anastomoses and to explore ways to promote repair under these conditions.

Design: Seven-day, prospective randomized experimental trial.

Setting: Animal research laboratory.

Animals: Male young-adult Wistar rats after resection and anastomosis of both ileum and colon.

Interventions: Random assignment to groups receiving placebo, daily fluorouracil (20 mg/kg per day, intraperitoneally), daily fluorouracil plus retinol palmitate (5000 IU/kg per day, orally), daily fluorouracil plus interleukin-2 (2×10^6 IU/kg per day, subcutaneously), or daily fluorouracil plus granulocyte macrophage colony-stimulating factor on the first 4 days after operation (20 μg/kg per day, intraperitoneally).

Main Outcome Measures: Anastomotic bursting pressure, breaking strength, hydroxyproline content, and ex vivo collagen synthetic capacity.

Results: Administration of fluorouracil decreased anastomotic breaking strength by more than 40% and caused a shift in bursting site from outside to within the suture line. It also lowered anastomotic hydroxyproline content. The capacity for collagen synthesis, which was greatly enhanced in 4-day-old anastomoses from the control group, was significantly ($P<.05$) and specifically reduced. Concomitant administration of retinol resulted in restoration of strength and hydroxyproline content, particularly in the ileum. Interleukin-2 and granulocyte macrophage colony-stimulating factor did not improve fluorouracil-suppressed repair: both wound strength andcollagen content were similar in the fluorouracil, fluorouracil/interleukin-2, and fluorouracil/granulocyte macrophage colony-stimulating factor groups.

Conclusion: Intraperitoneal administration of fluorouracil, delivered from the day of operation onward, severely reduces anastomotic strength at the end of the first postoperative week. This negative effect may be prevented by oral administration of retinol.

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IN THE WESTERN world, colorectal cancer is the second most frequent malignant neoplasm after lung cancer. More than half the patients with this disease will die of it as a consequence of locoregional or distant spread. While surgery remains the only curative treatment modality for early-stage disease, the most important prognostic feature noted to date in colorectal cancer is the surgical pathologic stage of the tumor resected. At least 90% to 95% of patients with Dukes' stage A or B_1 carcinoma of the colon are cured by surgical resection alone. However, patients with stage B_2 disease have a 30% to 70% chance of 5-year survival after surgical therapy alone, and patients with stage C disease have only a 10% to 50% chance.[1] In these patients, who represent 60% to 70% of the total patient population presenting with colorectal cancer, adjuvant therapy is highly indicated. Fluorouracil is the recommended standard treatment and has remained the building block for adjuvant trials.[2]

Adjuvant therapy in colorectal cancer routinely is withheld until weeks after surgery. However, there exists an excellent rationale to start therapy in the immediate postoperative period.[3] Theories of tumor-cell kinetics and drug resistance predict that cancer cells are more susceptible to anticancer therapy when the tumor burden is small.[4] Numerous animal model studies have demonstrated that a primary tumor can

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MATERIALS AND METHODS

ANIMALS

Seventy male outbred Wistar/Cpb:WU rats, weighing between 200 and 300 g, were used. They were housed with two animals per cage and had free access to water and standard laboratory chow, which contained 21.7 IU retinol palmitate per gram (diet AM II, Hope Farms, Woerden, the Netherlands).

For the first experiment, in which anastomotic strength and hydroxyproline content were measured, the animals were randomly divided into five groups: a control group (n=11), a fluorouracil group (n=11), and three groups (n=10 each) that received fluorouracil plus retinol, GM-CSF, or interleukin-2. All rats were killed 7 days after surgery.

The second experiment, measuring collagen synthesis, consisted of three groups of animals (n=6 each): a control group, a fluorouracil group, and a group that received fluorouracil plus retinol. These rats were killed 4 days after surgery.

The study was approved by the Animal Ethics Review Committee of the Faculty of Medicine, University of Nijmegen (the Netherlands).

DRUG ADMINISTRATION

Fluorouracil was given intraperitoneally in a dosage of 20 mg/kg of body weight. This is the same dosage used before[12] and represents the highest dosage that, in combination with surgery, did not result in a significant mortality rate. Fluorouracil was administered once a day, immediately after surgery and on the next 6 days, at a concentration of 1 mg/mL of saline solution.

Retinol palmitate was given orally (by gavage) from the day before surgery onward in a dosage of 5000 IU/kg per day. Recombinant murine GM-CSF was dissolved in sodium phosphate, 20 mmol/L, pH 7.5; sodium chloride, 0.15 mol/L; and rat albumin, 5 mg/mL. It was administered intraperitoneally immediately after surgery and on the next 3 days in a dosage of 5 μg (2.5 mL of the solution) per day. Recombinant human interleukin-2 was reconstituted in water and diluted with 5% wt/wt dextrose in water. It was given subcutaneously (0.25 mL of solution) once a day from the day of surgery onward in a dosage of 2×10^6 IU/kg of body weight.

In previous experiments that used the current model for intestinal healing, we established that anastomotic repair is not affected by daily administration of placebo solutions, either by gavage or by intravenous, intraperitoneal, or subcutaneous injection (T.H., unpublished data, 1990 and 1992). For this reason the animals in the present control groups received intraperitoneal saline solution daily.

OPERATIVE PROCEDURE

After an intraperitoneal injection of pentobarbital sodium, a midline incision was made, and 1 cm of both small and large bowel were resected at 15 cm proximal to the ileocecal junction and 3 cm proximal to the rectal peritoneal reflection, respectively. Continuity was restored microsurgically by the construction of an inverted one-layer seromuscular end-to-end anastomosis with eight interrupted sutures of 8-0 monofilament material (Ethicon, Somerville, Mass). The abdomen was closed in two layers with a continuous 3-0 silk suture for the fascia and with staples for the skin.

inhibit the growth of metastatic deposits and that its removal can increase the growth rate of metastases.[5] Experimental data indicate that the efficiency of adjuvant therapy is inversely proportional to the time interval between surgery and therapy. Although, so far, limited information exists to support timing decisions in human neoplasms, the considerations mentioned above support the thesis that treatment with anticancer agents should begin at the day of surgery or as soon as possible thereafter.[3,6,7]

Patients will present with recurrent disease at the operative site or the peritoneal surface and/or with hepatic metastases and systemic metastases. Although percentages in the literature vary widely, local recurrence is high.[8] Ideally, an adjuvant surgical therapy should be effective at all sites of recurrence, both locoregional and systemic. However, elimination of recurrence at a particular anatomical site may benefit a significant proportion of patients in terms of survival or quality of life. Regional administration of cytostatic agents is expected to reduce systemic passage and thus general toxic effects, allowing higher local concentrations. This fact is important in view of the fact that the fluorouracil response rate may depend strongly on dose intensity.[9]

A number of clinical studies using immediate (0 to 4 days) postoperative intraperitoneal administration of fluorouracil are in progress,[10] and a current European Organization for Research and Treatment of Cancer Trial (protocol 40911) has one arm in which patients are treated intraperitoneally with fluorouracil in the perioperative period (Gastrointestinal Tract Cancer Cooperative Group, unpublished data, 1992). In an animal model of colon cancer, intraperitoneal administration of fluorouracil prevents peritoneal and hepatic metastasis.[11]

As interest in immediate postoperative local and/or systemic adjuvant fluorouracil therapy rapidly increases, it becomes essential to delineate its hazards for anastomotic healing and to develop strategies to prevent negative effects because leakage of intestinal anastomoses is a potentially devastating surgical complication.

It was shown before that administration of fluorouracil on the day of surgery and the next 2 days does not significantly reduce strength in experimental intestinal anastomoses.[12] Recently, Graf et al[13] reported that prolongation of the fluorouracil therapy impaired the healing of colonic anastomoses. The present study describes the effects of a 7-day course of intraperitoneal administration of fluorouracil on repair of both ileal and colonic anastomoses in the rat. In addition, we examine the potential beneficial effects of retinol palmitate, granulocyte macrophage colony-stimulating factor (GM-CSF), and interleukin-2.

RESULTS

Only one animal (from the fluorouracil/retinol group) died prematurely, owing to faulty oral administration of the drug.

All animals lost weight after surgery. In all groups, average weight loss was 9% of body weight at the first day

ANALYTICAL PROCEDURES

The rats were killed by an intraperitoneal overdose of pentobarbital sodium. After opening the abdominal wound and identifying the anastomoses, the adhesions were cut as far as possible without injuring the intestine. An intestinal segment with the anastomosis in the middle was removed, with the sutures left in place. This segment was attached to an infusion pump filled with methylene blue–stained saline solution. The pressure was raised with an infusion rate of 4 mL/min and recorded graphically. Both the bursting pressure (the maximum pressure recorded immediately before sudden loss of pressure) and the site of rupture were noted. Thereafter, the segment was placed in a tensiometer, and the breaking strength was recorded. Thus, both the bursting pressure and the breaking strength were measured in the same anastomotic segment. The validity of this procedure had been confirmed in a pilot experiment. In two series of animals, the breaking strength was measured directly or after the procedure to obtain the bursting pressure. Similar values for the average breaking strength were obtained in both series (B. M. de Man and T.H., unpublished data, 1992).

The anastomotic segment was then cleaned from the surrounding tissue, and a 5-mm segment with the suture line in the middle was collected. The samples were frozen immediately and stored in liquid nitrogen until processing. After weighing, the samples were pulverized and lyophilized, and the hydroxyproline content was measured as described before.[14]

In the second experiment, collagen synthetic capacity in control segments (removed at operation) and anastomotic tissue were quantitated by measuring the incorporation of proline into collagenase digestible protein (CDP) according to a procedure validated before for rat intestinal tissue.[15,16] Briefly, freshly collected tissue explants of 1 to 2 mm^2 were incubated in a medium containing tritiated proline for 3 hours, and the radioactivity incorporated into total protein was measured. Subsequently, to determine proline incorporation into collagen, excess purified collagenase was added. The radioactivity in the supernatant represents CDP as a measure of the amount of collagen synthesized. Subtraction of the radioactivity in the CDP fraction from that in total protein yields the incorporation into noncollagenous protein (NCP). The relative collagen synthesis (RCS) was calculated with a formula[17] that takes into account the enrichment of proline in collagen compared with other proteins:

$$\text{Percentage of RCS} = [\text{CDP}/[(\text{NCP} \times 5.4) + \text{CDP}]] \times 100\%$$

Incorporation is expressed on the basis of sample wet weight, DNA[18] content, or protein[19] content.

STATISTICAL ANALYSIS

The main questions to be answered were the following: are anastomotic strength and hydroxyproline levels reduced in the four groups receiving fluorouracil when compared with the control group, and second, are these variables improved in the fluorouracil/retinol, fluorouracil/GM-CSF, and fluorouracil/interleukin-2 groups when compared with the fluorouracil group? To correct for the fact that multiple comparisons were made, pairwise comparisons were done using a level of significance of $\alpha' = 2\alpha/k$, where k is the total number of pairwise comparisons. Thus, differences between groups (**Figure 1** and **Figure 2**) were considered significant ($\alpha = .05$) at $P < \alpha'$, where $\alpha' = .014$. The test used was a one-tailed Wilcoxon test.

after surgery. Thereafter, animals in the control group started to gain weight again, and at death, their average weight was 6% over the weight at surgery. Weight gain was retarded in the fluorouracil group (average weight at day 7 was 94% of weight at surgery, $P = .0002$ vs control group), whereas rats in the fluorouracil/GM-CSF and fluorouracil/interleukin-2 groups lost progressively more weight, the average weight at day 7 being only 85% ($P = .0007$ vs fluorouracil group) and 84% ($P = .0006$ vs fluorouracil group), respectively, of the weight at surgery. In the group that received retinol in addition to fluorouracil, average weight at day 7 was 98% of the initial weight ($P = .0004$ and $P = .08$ vs control and fluorouracil group, respectively).

Average anastomotic breaking strength is depicted in Figure 1. Administration of fluorouracil resulted in significantly reduced anastomotic strength: breaking strength was lowered by 41% in ileal anastomoses and by 44% in the colonic anastomoses. Addition of GM-CSF or interleukin-2 did not improve anastomotic strength. If the animals were given retinol and fluorouracil, the resulting breaking strength was significantly higher than that in rats receiving fluorouracil only. Although mean values were still lower than those of the control group, the difference was not statistically significant.

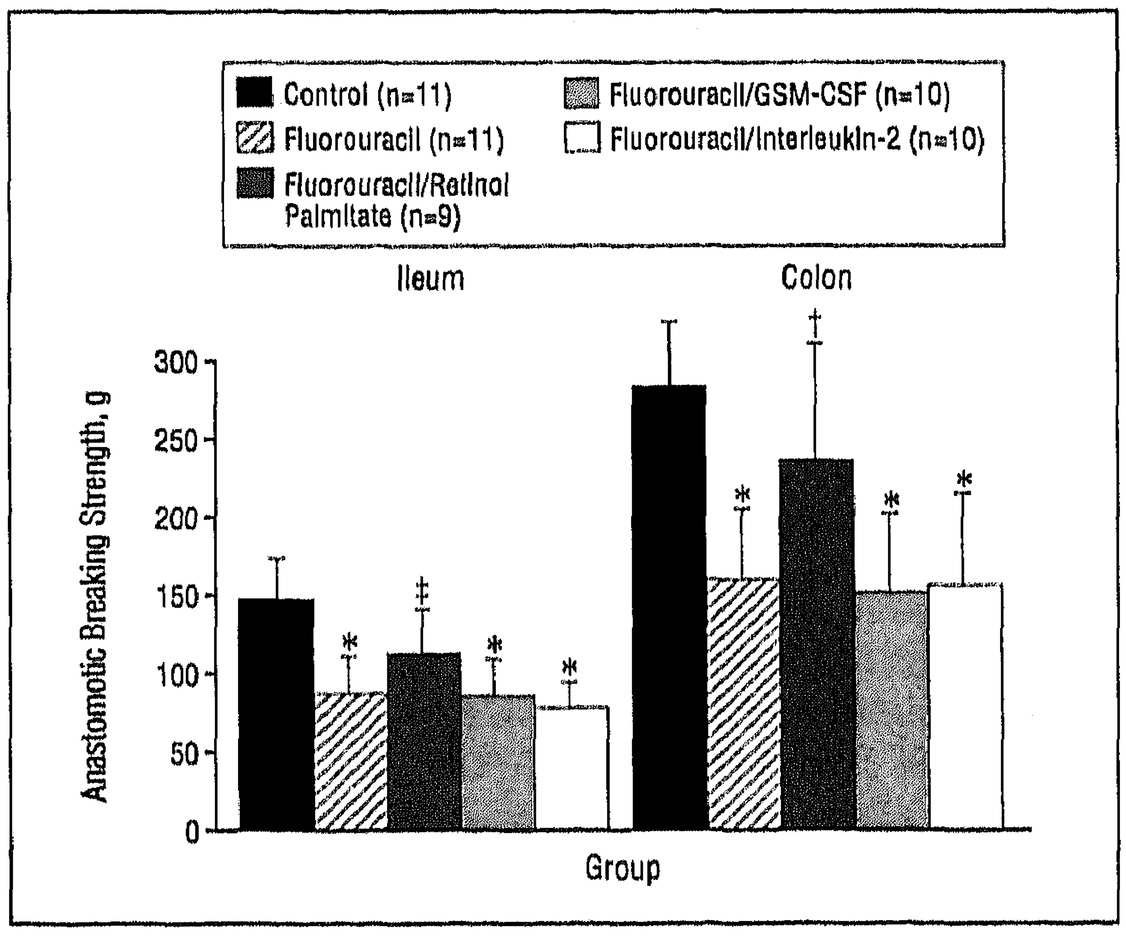

Figure 1. *Anastomotic breaking strength. Bars represent mean+SD values. Asterisk denotes significantly ($P < \alpha'$, where $\alpha' = .014$ [see "Methods" section]) different from control group; dagger, significantly different from fluorouracil group; and double dagger, nearly significantly ($\alpha' < P < 2\alpha'$) different from fluorouracil group.*

Figure 3 shows the values for the bursting pressure obtained from the individual animals. In some animals, notably those from the fluorouracil/interleukin-2 group, it proved to be technically difficult to prepare the anastomotic segment for this analysis. As a consequence of iatrogenic damage, no bursting pressure could be measured in three ileal and two colonic anastomoses from this group and in one ileal anastomosis from the fluorouracil group. In the control group, the bursting site was always

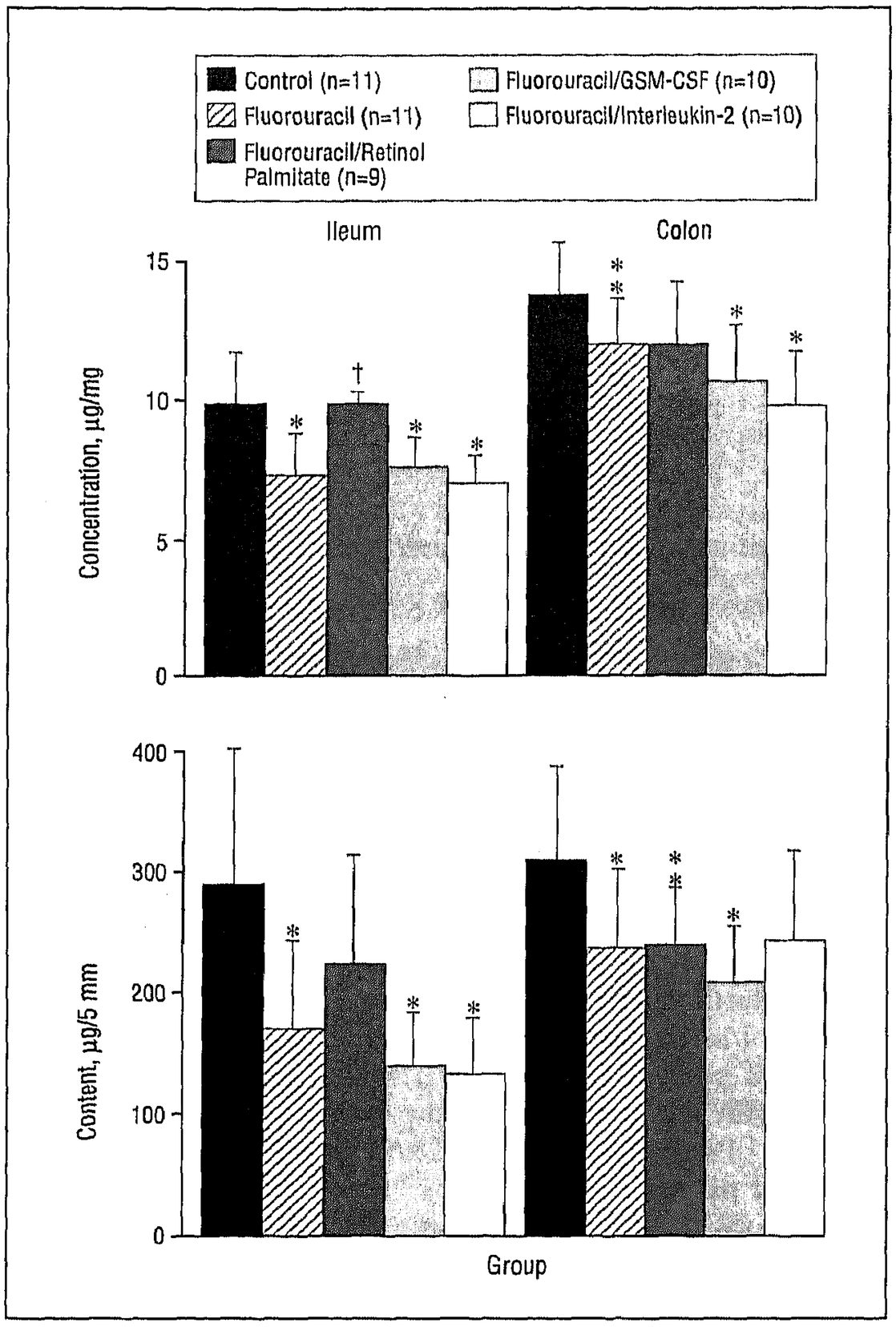

Figure 2. *Anastomotic hydroxyproline concentration and content. Bars represent mean+SD values. Asterisk denotes significantly (P<α′, where α′=.014 [see "Methods" section]) different from control group; dagger, significantly different from fluorouracil group; and double asterisks, nearly significantly (α′<P<2α′) different from control group.*

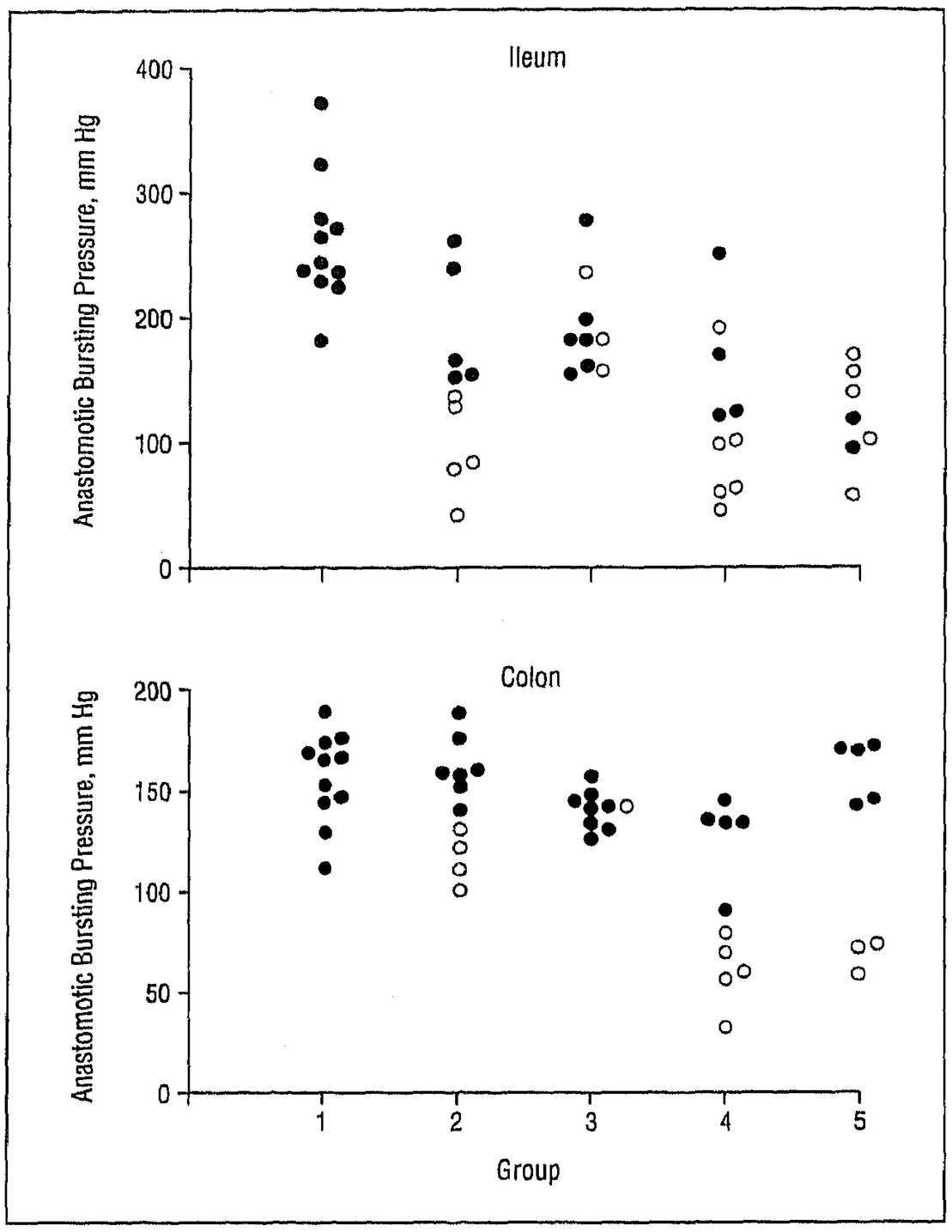

Figure 3. *Anastomotic bursting pressure. Symbols represent values from individual animals. Open circles indicate rupture within suture line; closed circles, rupture outside suture line. Group 1 is the control group (n=11); group 2, fluorouracil group (n=11); group 3, fluorouracil/retinol palmitate group (n=9); group 4, fluorouracil/granulocyte macrophage colony-stimulating factor group (n=10); and group 5, fluorouracil/interleukin-2 group (n=10).*

outside the suture line, meaning that the actual value no longer reflects wound strength because the anastomoses had grown stronger than the adjacent tissue. This was not the case in the other groups. In the fluorouracil group, rupture occurred within the anastomotic area in nine of 21 cases, confirming the reduction of anastomotic strength also observed by measuring the breaking strength. A similar phenomenon was observed in the groups that received additional GM-CSF or interleukin-2. Again, addition of retinol appeared to improve strength: in this group, only four of 18 wounds ruptured within the suture line. This effect is also apparent if the mean anastomotic bursting pressures were calculated using only those cases in which rupture occurred within the anastomosis. For instance, for ileum, these mean±SD values were 94±38 mm Hg (n=5) in the fluorouracil group and 190±40 (n=3), 93±52 (n=6), and 123±44 (n=5) mm Hg in the fluorouracil/retinol, fluorouracil/GM-CSF, and fluorouracil/interleukin-2 groups, respectively.

Tissue hydroxyproline levels were quantified as a measure of collagen levels, and the average values for hydroxyproline concentration and content in a 5-mm anastomotic segment are shown in Figure 2. In the fluorouracil group, anastomotic hydroxyproline concentration and content were lower than in the control group. The same was true for the fluorouracil/GM-CSF group and the fluorouracil/interleukin-2 group. Addition of retinol appeared to prevent the decrease in hydroxyproline concentration and to

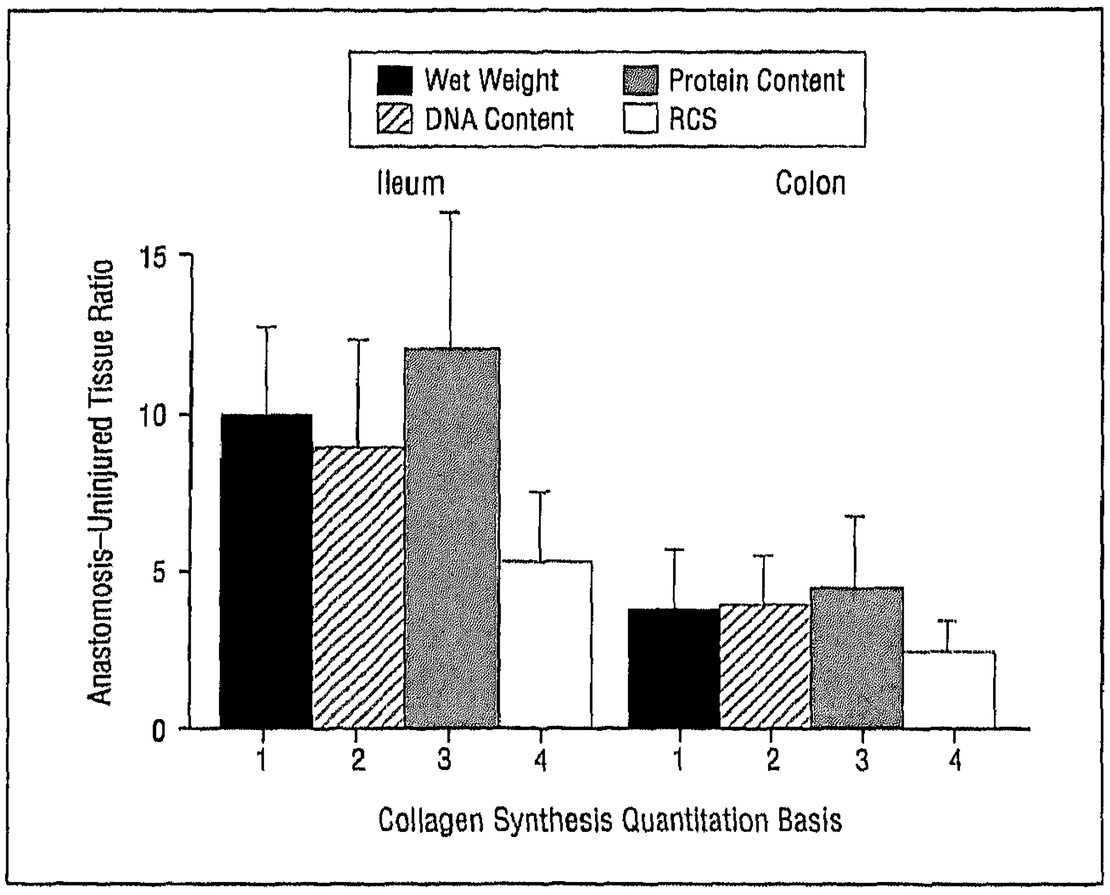

Figure 4. *Increased collagen synthetic capacity in anastomoses. Bars represent the mean+SD ratio (n=6) between ex vivo collagen synthesis measured in anastomotic tissue collected 4 days after surgery and uninjured intestine collected at surgery in animals from the control group. Values are calculated if synthesis is quantitated on the basis of wet weight, DNA content, or protein content or as relative collagen synthesis.*

Table 1. Ex Vivo Synthesis of Collagen and Noncollagen Protein in Ileal Anastomoses*

	Control Group	Fluorouracil Group	Fluorouracil/ Retinol Palmitate Group
Collagen			
DNA, dpm/μg	182±70	97±22†	82±17†
Wet weight, dpm/mg	440±124	266±51†	225±60†
Protein, dpm/mg	14 481±5302	7261±1840†	7978±1184†
Percentage of RCS	1.53±0.64	0.76±0.44	0.74±0.18
Noncollagen			
DNA, dpm/μg	2216±272	2794±777	2065±326
Wet weight, dpm/mg	5622±1368	7782±3305	5570±856
Protein, dpm/mg	177 929±25 090	206 013±65 876	201 810±32 458

**Explants from anastomotic tissue were collected 4 days after surgery and incubated for 3 hours with 166.5 kBq tritiated proline. Collagen synthesis is expressed as radioactivity in collagenase digestible protein and as percentage of relative collagen synthesis (RCS). Noncollagen protein synthesis is expressed as radioactivity in noncollagenous protein. Data represent average values (±SD) from six animals. dpm indicates disintegrations per minute.*

†Significantly different from control group (Wilcoxon two-sample test).

limit the lowering of hydroxyproline content, induced in ileal anastomoses by fluorouracil.

We also compared the collagen synthetic capacity, measured ex vivo in tissue explants, in the control, fluorouracil, and fluorouracil/retinol groups. Anastomotic collagen synthetic capacity was increased greatly 4 days after surgery. **Figure 4** shows that, in the control group, absolute collagen synthesis was enhanced approximately 10-fold in ileal anastomoses and fourfold in colonic anastomoses. Although synthesis of NCP was also stimulated (data not shown), the increase was relatively specific for collagen, as indicated by the considerable enhancement of the percentage of RCS.

The average anastomotic synthetic capacity in ileum and colon of the various groups is given in **Table 1** and **Table 2**, respectively. Clearly, administration of fluorouracil resulted in a specific and significant reduction of collagen production capacity. This effect was seen irrespective of whether synthesis was expressed on the basis of wet weight, DNA content, or protein content. The capacity to produce NCP was hardly affected. Administration of retinol together with fluorouracil did not change the synthesis in ileal anastomoses compared with administration of fluorouracil alone. In contrast, retinol appeared to stimulate collagen synthetic capacity in the colonic anastomoses. If collagen synthesis was expressed on the basis of DNA or protein content or as RCS, values in the fluorouracil/retinol group were significantly higher than in the fluorouracil group and no longer significantly different from those observed in the control group. In addition, the production capacity for NCP appeared to be significantly lower in the fluorouracil/retinol group than in the control group.

COMMENT

Trials for adjuvant chemotherapy for colorectal cancer began in the 1950s. Until now, disappointing results have been obtained despite enormous preclinical and clinical research efforts. For more than 30 years, fluorouracil has been the mainstay of therapy, and today it remains the most effective single agent in the treatment of this disease, although a meta-analysis of phase III randomized control trials showed only limited benefit to its use.[20] Much attention is directed at examining methods of enhancing fluorouracil efficacy through biochemical modulation. However, current efforts to improve outcome are also directed at optimizing fluorouracil treatment schedules with respect to dosage and timing and route of administration.

In relation to the aspect of timing, interest in immediate postoperative fluorouracil therapy is growing. In an effort to reduce the incidence of hepatic metastasis, several recent trials have explored the efficacy of perioperative portal vein infusion of fluorouracil.[7] Also, a number of clinical studies using perioperative intraperitoneal administration of fluorouracil are in train (Glimelius and Påhlman[10] and the Gastrointestinal Tract Cancer Cooperative Group, unpublished data, 1992). Thus, research into the effects of perioperative fluorouracil on the healing of intestinal anastomoses is highly indicated.

It is to be expected that the occurrence of any negative effect of fluorouracil administration on anastomotic healing will depend on the time of administration with respect to surgery. Probably, postponement of therapy until after the cellular phase of healing, when the wound has gained sufficient strength, will prevent any danger for anastomotic insufficiency. If treatment is started immediately after surgery, the chances for complications may increase. Still, Hillan et al[21] concluded that early postoperative intraperitoneal administration (at 0 to 4, 3 to 7, or 7 to 12 days after surgery) of fluorouracil does not impair the healing of experimental colonic anastomoses. However, a major drawback of their study is the fact that anastomotic strength was assessed only after 14 days, whereas eventual detrimental effects in the first week when strength is still low under normal healing conditions are of more potential significance. It was found that three intraperitoneal doses of fluorouracil (at 0 to 2 days after surgery) slightly but not significantly lowered anastomotic breaking strength and collagen content of 3- or 7-day-old intestinal anastomoses.[12] Our data show that continuation of treatment may result in a severe reduction of both anastomotic bursting pressure and breaking strength at 7 days after surgery. This outcome agrees with the recent findings of Graf et al,[13] who reported reduced breaking strength of colonic anastomoses after a similar fluorouracil regimen.

Assuming that wound strength is ultimately decided by collagen (either its quantity or its quality),

Table 2. Ex Vivo Synthesis of Collagen and Noncollagen Protein in Colonic Anastomoses*

	Control Group	Fluorouracil Group	Fluorouracil/ Retinol Palmitate Group
Collagen			
DNA, dpm/μg	201±90	102±36†	151±32‡
Wet weight, dpm/mg	870±436	423±154†	529±91
Protein, dpm/mg	20 667±9999	9914±2936†	18 243±3536‡
Percentage of RCS	2.18±0.95	1.30±0.41	2.29±0.44‡
Noncollagen			
DNA, dpm/μg	1690±274	1440±307	1175±80†
Wet weight, dpm/mg	7274±1710	6033±1420	4190±504†
Protein, dpm/mg	177 248±24 655	141 355±23 873†	143 362±15 244†

**Explants from anastomotic tissue were collected 4 days after surgery and incubated for 3 hours with 166.5 kBq tritiated proline. Collagen synthesis is expressed as radioactivity in collagenase digestible protein and as percentage of relative collagen synthesis (RCS). Noncollagen protein synthesis is expressed as radioactivity in noncollagenous protein. Data represent average values (±SD) from six animals. dpm indicates disintegrations per minute.*
†Significantly different from control group (Wilcoxon two-sample test).
‡Significantly different from fluorouracil group (Wilcoxon two-sample test).

changes in collagen metabolism are expected to affect anastomotic strength. The capacity for anastomotic collagen synthesis in the fluorouracil group was only half that in the control group. This may explain the reduced anastomotic hydroxyproline content, although we cannot yet preclude the possibility of concomitant increased degradation of existing collagen fibers. It thus seems likely that a diminished presence of collagen contributes to loss of strength, as observed in the fluorouracil group, although changes in its quality, eg, the degree of cross-linking, may also play a role.

Wound collagen synthesis is primarily a task for the fibroblast. Suppressed synthesis by fluorouracil administration may be due to a direct toxic effect on fibroblasts or to suppression of regulatory inflammatory cells like T lymphocytes and macrophages. The latter play a central role in the repair sequence.[22,23] Chemotherapy is known to decrease the number of monocytes. In earlier experiments, indications that a perioperative regimen of cytostatic drugs, including fluorouracil, delays the appearance of macrophages in the anastomotic area were found.[24] Because recombinant murine GM-CSF has been reported to induce monocytosis and to increase the number of tissue macrophages,[25,26] it seemed worthwhile to investigate whether administration of this compound could ameliorate fluorouracil-impaired healing. However, when given for 4 consecutive days from the day of surgery onward in a dose that had been proven effective in eliciting the desired hemapoietic response in rodents,[25,26] it did not improve healing. The animals in the fluorouracil/GM-CSF group lost progressively more weight than did those in the fluorouracil group, and anastomotic repair was not stimulated. If anything, anastomotic bursting pressure and hydroxyproline levels were reduced even further.

Major surgery impairs the cellular immune response. It has been suggested that fluorouracil-based chemotherapy is associated with suppression of immune function.[27] Thus, perioperative fluorouracil therapy might result in depressed function of macrophages and T lymphocytes. Stimulation of these cells might improve fluorouracil-suppressed healing. Interleukin-2, which supports the growth and function of activated T cells and macrophages, has been reported to improve cutaneous healing in rats treated with doxorubicin hydrochloride (Adriamycin).[28] Interleukin-2 reverses the deleterious effects, supposedly caused by immunosuppression, of blood transfusion on anastomotic healing.[29] Perioperative immunotherapy with interleukin-2 has been suggested as a means to improve the cellular immune system of patients undergoing surgery for colorectal cancer.[30] Still, daily administration of interleukin-2 to the fluorouracil-treated rats in a dose that effectively stimulated transfusion-suppressed healing did not improve anastomotic healing: wound strength and collagen content were similar in the fluorouracil and fluorouracil/interleukin-2 groups.

Retinol is one of the retinoids that have a profound impact on many biological functions. The apparent antitumor effects of retinoids have generated renewed enthusiasm for their application in clinical studies for oncologic therapeutic indications.[31] The healing-promoting capacity of retinol, especially under conditions that retard repair, has been reported repeatedly. It has been shown to improve various aspects of healing in experimental cutaneous wounds in which repair was suppressed by steroids,[32] induced diabetes,[33] irradiation,[34] or tumor implantation.[35] With regard to anastomotic healing, Winsey et al[36] found that retinol supplementation mitigated the negative effects of preoperative irradiation on the bursting pressure and hydroxyproline concentration in 7-day-old rat colonic anastomoses. Interpretation of their data is hampered because no data on bursting site were supplied. The same is true for a recent report that showed that high-dose retinol therapy reversed the inhibitory effects of long-term administration of corticosteroids on the healing of ileal and colonic anastomoses.[37]

Our findings indicate that administration of retinol may be useful in preventing the loss of early anastomotic strength that is induced by the use of cytostatic drugs administered from the day of surgery onward. Although all animals did receive retinol in their standard diet (up to an estimated 800 IU/kg per day), administration of additional retinol enhanced anastomotic breaking strength in animals receiving fluorouracil. It also improved the bursting pressure, reducing the frequency of bursting that occurred within the suture line. In those anastomotic segments in which the bursting site was within the anastomosis, the actual bursting pressures measured were higher in the fluorouracil/

etinol group than in the fluorouracil group. It is conceivble that augmentation of the retinol dose may further nhance its efficacy. The dose given, 5000 IU/kg per day, s well below those administered to reverse the negative efects of corticosteroids on wound repair.[37,38]

The mechanism by which retinol antagonizes the effect of fluorouracil remains to be elucidated. Retinol adminstration may enhance the early inflammatory reaction to vounding, increasing the number of monocytes and macophages at the injury site.[39] Speculation also exists regardng stimulation of collagen synthesis and inhibition of colagen degradation (Weinzweig et al[35] and Phillips et al[37]). Ve have found no consistent support for a stimulation of ollagen synthesis. In ileal anastomoses, the collagen synhetic capacity was similar in the fluorouracil and fluorouracil/etinol groups, whereas the average anastomotic hydroxyroline content appeared to be increased in the fluorouracil/etinol group (and not significantly lower than in the control roup). In the colon, the collagen synthetic capacity was higher n the fluorouracil/retinol group than in the fluorouracil group, vhereas the hydroxyproline content was similar in both roups. It should be emphasized that the hydroxyproline ontent was measured in a 5-mm segment containing unnjured tissue next to the suture line. Thus, small and local hanges in either collagen degradation or collagen synthesis may remain undetected. Whatever mechanism is responsible for the gain in wound strength, the beneficial effects bserved in the present study seem to be of potential clinical interest and warrant further investigation.

In summary, daily intraperitoneal administration of luorouracil from the day of surgery onward considerably educed anastomotic strength after 7 days. This may have been caused by a reduction in collagen synthetic capacty within the wound area. Loss of strength could not be revented by additional administration of GM-CSF or nterleukin-2, but the negative effects of fluorouracil were meliorated by oral administration of retinol.

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